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software for automated genotype calling. Each of the relevant gene regions are PCR amplified from purified genomic DNA in a single reaction using the following oligonucleotide primer designs:

			·
5	Gene Primer	Sequence	(5' - 3')
	RHD Exon 4	RHDe4S	AGACAAACTGGGTATCGTTGC (SEQ ID NO: 1)
		RHDe4A	ATCTACGTGTTCGCAGCCT (SEQ ID NO: 2)
10	RHD Exon 9	RHDe9\$	CCAAACCTTTTAACATTAAATTATGC (SEQ ID NO: 3)
	•	RHDe9A	TTGGTCATCAAAATATTTAGCCTC (SEQ ID NO: 4)
	RHCE Exon 2	RHCEe2S	TGTGCAGTGGGCAATCCT (SEQ ID NO: 5)
		RHCEe2A	CCACCATCCCAATACCTG (SEQ ID NO: 6)
15	RHCE Exon 5	RHCEe55	AACCACCCTCTCTGGCCC (SEQ ID NO: 7)
		RHCEe5A	ATAGTAGGTGTTGAACATGGCAT (SEQ ID NO: 8)
	GYPB Exon 4	GYPBe4S	ACATGTCTTCTTATTTGGACTTAC . (SEQ ID NO: 9)
20		GYPBe4A	TTTGTCAAATATTAACATACCTGGTAC (SEQ ID NO: 10)
	KEL Exon 6	KELe6S	TCTCTCTCTTTAAAGCTTGGA (SEQ ID NO: 11)
		KELe6A	AGAGGCAGGATGAGGTCC (SEQ ID NO: 12)
25	KEL Exon 8	KELe8S	AGCAAGGTGCAAGAACACT (SEQ ID NO: 13)
		KELe8A	AGAGCTTGCCCTGTGCCC (SEQ ID NO: 14)
	FY Promoter	FYproS	TGTCCCTGCCCAGAACCT (SEQ ID NO: 15)
		FYproA	AGACAGAAGGGCTGGGAC (SEQ ID NO: 16)
30	FY Exon 2	FYe2S	AGTGCAGAGTCATCCAGCA (SEQ ID NO: 17)

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•		FYe2A	TTCGAAGATGTATGGAATTCTTC SEQ ID NO: 18)
,	JK Exon 9	JKe95	CATGAACATTCCTCCCATTG (SEQ ID NO: 19)
5		JKe9A	TTTAGTCCTGAGTTCTGACCCC (SEQ ID NO: 20)
	DI Exon 18	DIe19S	ATCCAGATCATCTGCCTGG (SEQ ID NO: 21)
		DIe19A	CGGCACAGTGAGGATGAG (SEQ ID NO: 22)
10	GP3A	GP3Ae3\$	ATTCTGGGGCACAGTTATCC (SEQ ID NO: 23)
	•	GP3Ae3A	ATAGTTCTGATTGCTGGACTTCTC (SEQ ID NO: 24)

The above primer pairs comprise the corresponding forward and reverse primers, and may be referred to herein as SEQ ID NOs 1-24.

Multiplexed single nucleotide primer extension is performed using the following 5' tagged extension primers:

- RHD Exon 4 GTGATTCTGTACGTGTCGCCGTCTGATCTTTATCCTCCGTTCCCT (SEQ ID NO: 25)
  - RHD Exon 9 GCGGTAGGTTCCCGACATATTTTAAACAGGTTTGCTCCTAAATCT (SEQ ID NO: 26)
  - RHCE Exon 2 GGATGGCGTTCCGTCCTATTGGACGGCTTCCTGAGCCAGTTCCCT (SEQ ID NO: 27)
- 25 RHCE Exon 5 CGACTGTAGGTGCGTAACTCGATGTTCTGGCCAAGTGTCAACTCT (SEQ ID NO: 28)
  - GYPB Exon 4 AGGGTCTCTACGCTGACGATTTGAAATTTTGCTTTATAGGAGAAA (SEQ ID NO: 29)
- KEL Exon 6 AGCGATCTGCGAGACCGTATTGGACTTCCTTAAACTTTAACCGAA (SEQ ID NO: 30)
  - KEL Exon 8 AGATAGAGTCGATGCCAGCTTTCCTTGTCAATCTCCATCACTTCA (SEQ ID NO: 31)

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- FY Promoter GACCTGGGTGTCGATACCTAGGCCCTCATTAGTCCTTGGCTCTTA (SEQ ID NO: 32)
- **ACGCACGTCCACGGTGATTT**GGGGGCAGCTGCTTCCAGGTTGGCA FY Exon 2 (SEQ ID NO: 33)
- 5 JK Exon 9 CGTGCCGCTCGTGATAGAATAAACCCCAGAGTCCAAAGTAGATGT (SEQ ID NO: 34)
  - DI Exon 19 GGCTATGATTCGCAATGCTTGTGCTGTGGGTGGTGAAGTCCACGC (SEQ ID NO: 35)
- GP3A Exon 3 AGAGCGAGTGACGCATACTTGGGCTCCTGTCTTACAXGCCCTGCCTC 10 (SEQ ID NO: 36)

The above probes may be referred to herein as SEQ ID NOs 25-36. The DNA bases are represented by their single letter equivalents (A,C,G or T) and the letter X represents 15 a C3 (phosphoramidite) spacer between the two adjacent DNA bases.

In this embodiment, the 12 bolded nucleotides in the region of the extension probes are hybridized to a complementary DNA sequence that has been micro-arrayed onto

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The PCR primers pairs in Table 1 represent sites. sequences complementary to DNA regions containing SNPs of interest; of which the exact sequences of each primer pair and mixture of primer pairs have been specifically optimized to amplify genomic DNA of interest as a mixture of 12 primer pairs. Although noted above, Table 2 further summarizes 12 novel extension primers specifically used together to detect the nucleotides of blood group and platelet antigen or HPA SNPs, simultaneously. The extension primers represent a group of 12 novel nucleotide 10 sequences, of which each are a combination of: 1) a unique 5' region necessary to direct hybridization to a microarrayed tag located in a specific spot in each microplate well, and 2) a 3' region complementary to and adjacent to a 15 SNP of a PCR-amplified DNA region containing the SNP of interest.

Table 1. The FCR primers used in the 12-pair multiplex PCR format for multiple SNP detection.

Antigen	SNP	Primer Name	Sequence 5'-3'	Product Target	Size (bp)	
RhD/RhCE	m /m	RHDe45	AGACAAACTGGGTATCGTTGC	RHD		
MITO VII CE	C/T	RHDe4A	ATCTACGTGTTCGCAGCCT	Exon 4	7.11	
RhD/RhCE	A/G	RHDe9S	CCAAACCTTTTAACATTAAATTATGC	RHD		
TOTO TOTO	A/G	RHDe9A	TTGGTCATCAAAATATTTAGCCTC	Exon 9	98	
RhC/Rhc	T/C	RHCEe25	TGTGCAGTGGGCAATCCT	RHCE	0.0	
TOTAL TOTAL	1/0	RHCEe2A	CCACCATCCCAATACCTG	Exon 2	90	
RhE/Rhe	C/G	RHCEe55	AACCACCCTCTCTGGCCC	RHCE	7.00	
MIG	C/G	RHCEe5A	ATAGTAGGTGTTGAACATGGCAT	Exon 5	107	
GYPBS/GYPBs	s T/C	GYPBe45	ACATGTCTTTCTTATTTGGACTTAC	GPYB	455	
arephyatens		GYPBe4A	TTTGTCAAATATTAACATACCTGGTAC	Exon 4	103	
K/k	T/C	KELe6S	TCTCTCTCTTTAAAGCTTGGA	KEL	5.45	
T/ YF		KELe6A	AGAGGCAGGATGAGGTCC	Exon 6	142	
Kp <sup>a</sup> /Kp <sup>b</sup>	T/C	KELe8S	AGCAAGGTGCAAGAACACT	KEL	100	
11 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1		KELe8A	AGAGCTTGCCCTGTGCCC	Exon 8	100	
Fy/Fy0	T/C	FYproS	TGTCCCTGCCCAGAACCT	Duffy	20	
1 4 / 1 A O		FYproA	AGACAGAAGGGCTGGGAC	Promoter	90	
Fy <sup>a</sup> /Fy <sup>b</sup>	G/A	FYe2S	AGTGCAGAGTCATCCAGCA	Duffy		
		FYe2A	TTCGAAGATGTATGGAATTCTTC	Exon 2	122	
Jkª/Jkʰ	G/A	JKe9S	CATGAACATTCCTCCCATTG	Kidd	7 7 0	
UK /UK		JKe9A	TTTAGTCCTGAGTTCTGACCCC	Exon 9	1.30	
Di <sup>a</sup> /Di <sup>b</sup>	T/C	DIe195	ATCCAGATCATCTGCCTGG	Diego	90	

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		Diel9A	CGGCACAGTGAGGATGAG	Exon 19	
			ATTCTGGGGCACAGTTATCC	GP3A	1114
HPA-la/b	T/C		ATAGTTCTGATTGCTGGACTTCTC	Exon 3	

The above primers correspond to SEQ ID NOs 1-24, respectively, as outlined herein above.

Table 1A. Additional Blood Group and Platelet Antigen SNPs for Clinically Relevant Antigens.

Antigen	SNP	Product Size Target (bp)
A/O GalNAc/Del	G/T	ABO Exon 6
A/B (GalNAc/Gal)	C/G	ABO Exon 7
A/B (GalNAc/Gal)	G/A	ABO Exon 7
A/B (GalNAc/Gal)	C/A	ABO Exon 7
A/B (GalNAc/Gal)	G/C	ABO Exon 7
M/N	G/A	MNS Exon 2
M/N	T/G	MNS Exon 2
MNS/MiI	C/T	MNS Exon 3
RHD/Weak D Type 1	T/G	RHD Exon 6
RHD/Weak D Type 2	G/C	RHD Exon 9
RHD/Weak D Type 3	C/G	RHD Exon 1
RHD/D nt602 Variants	Ç/G	RHD Exon 4
RHD/'DAR' Variant	T/C	RHD Exon 7
RHD/Weak D Type 5	C/A	RHD Exon 3
RHD/D <sub>el</sub>	G/A	RHD IVS3+1
RHD/D <sub>pl</sub>	G/T	RHD Exon 6
RHD/D <sub>el</sub>	G/A	RHD Exon 9
RHD/RHD\u00f3	А/Т	RHD Exon 4
RHCE/RhC	T/C	RHCE IVS2+1722

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Each antigen listed on the left represents a blood genotype single nucleotide HPA the Or and group polymorphism (SNP). Some genotypes are evaluated using more than one SNP because they differ by more than one Each PCR primer pair consists of a sense nucleotide. (Primer Name ending in S) and antisense (Primer Name ending in A) oligonucleotide (Sequence 5'-3') designed to amplify the DNA region containing the SNP for the antigen of The target region (Product Target) and the interest. amplified fragment (Size (bp)) are shown on the right. Note that 12 SNPs are evaluated for 19 different blood group and platelet antigens because some antigens have more than one SNP. In some cases an A or G SNP is included since the complementary DNA strand can be evaluated as it will contain the T or C SNP of interest.

Table 2. Extension probes used to detect the nucleotides of blood group and HPA SNFs.

Мале	Sequence 5'-3'
RHD Exon 4	GTGATTCTGTACGTGTCGCCGTCTGATCTTTATCCTCCGTTCCCT
RHD Exon 9	GCGGTAGGTTCCCCGACATATTTTAAACAGGTTTGCTCCTAAATCT
RHCE Exon 2	GGATGGCGTTCCGTCCTATTGGACGGCTTCCTGAGCCAGTTCCCT
RHCE Exon 5	CGACTGTAGGTGCGTAACTCGATGTTCTGGCCAAGTGTCAACTCT
GYPB Exon 4	. AGGGTCTCTACGCTGACGATTTGAAATTTTGCTTTATAGGAGAAA
KEL Exon 6	AGCGATCTGCGAGACCGTATTGGACTTCCTTAAACTTTAACCGAA
KEL Exon 8	ACATAGAGTCGATGCCAGCTTTCCTTGTCAATCTCCATCACTTCA
FY Promoter	GACCTGGGTGTCGATACCTAGGCCCTCATTAGTCCTTGGCTCTTA
FY Exon 2	ACGCACGTCCACGGTGATTTGGGGGCAGCTGCTTCCAGGTTGGCA
JK Exon 9	CGTGCCGCTCGTGATAGAATAAACCCCAGAGTCCAAAGTAGATGT
Di Exon 19	GGCTATGATTCGCAATGCTTGTGCTGTGGGTGGTGAAGTCCACGC
GP3A Exon 3	AGAGCGAGTGACCCATACTTGGGCTCCTGTCTTACAXGCCCTGCCTC

The above probes correspond to SEQ ID NOs 25-36, respectively, as identified herein above. The DNA bases are represented by their single letter equivalents (A,C,G or T) and the letter X represents a C3 (phosphoramidite) spacer between the two adjacent DNA bases.

The present invention also provides novel hybrid probes, wherein the preferred probes are listed in Table 2, but limited to said listing. Each extension probe is

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automatically generated using the SNPStream Software Suite of MegaImage, UHTGetGenos and QCReview.

It should be noted that the specific steps associated with the protocol exemplified in Example 1 are not intended to limit the teachings and methods of the present invention to the specific above protocol. Example 1 is provided to specify a preferred method in accordance with the present invention wherein a plurality of blood group and HPA SNPs are simultaneously analysed in a ultra high throughput multiplex automated system for the determination of the specific accordingly the genotypes phenotypes and Accordingly, it should be understood by one therewith. skilled in the art that the steps of Example 1 may be varied provided that such variations yield the preferred results of 15 · the present invention.

#### RESULTS

#### GP3A Exon 3 SNP Scatter Plots.

The robotic UHT platform produces laser-fluorescence values for each sample which are represented in 'scatter' plots' for the operator to review. A sample scatter plot is 20 shown in Fig. 1 for the SNP analysis GP3A Exon 3, which represents the HPA-la and HPA-lb antigens. As can been seen in Fig. 1 and Fig. 4, results are graphed using logarithmic and XY scatter plots (upper right). Green O, orange [] or blue O sample designations represent CC, TC and TT SNP genotype calls, respectively, with corresponding graphical summaries appearing in the respective legends of each figure. No fluorescence represents an assay failure (FL) for that sample.

30 Scatter plots (as shown in Fig. 1 and Fig. 4) generated preferably using SNPStream software suite viewed through QCReview. It should be additionally noted that the present analysis is not limited to SNPstream or

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#### Appendix A

#### Genotype Results for updated 12 SNP CBS Panel

Shinerossia	ESTATE HONDON			C RHOPPING	ASSETUE DAYS
BB24401	FL	FL	FL	FL	FL
BB24402	TT	FL	CC	CC	TO
BB24407	TC	TT	TC	TC	TÇ
BB24408	TC	π	TC	TC	cc
BB24409	TC	TT	TC	TC	, LC
BB24410	TC	ii	TC	TC	ŤČ
BB24415	TC	TT	TÇ	TC	FL
BB24416	FL	FL	FL.	FL	FL.
	TC	† <del>-</del>	TÇ	FL	TČ
BB24417			TC	TC	CC
BB24420	TC	<b>TT</b>		TC	
BB24421	TC	TT	TC		ÇÇ TO
BB24422	TC	TT	TC ·	FĻ TC	TC TC
BB24423	TC	7 <b>7</b>	TC		TC TC
BB24424	TC	TT .	TC TC	FL	TC
BB24425	TC	· 7T	TC	TC TO	CC
BB24426	TC TO	TT	TC	TC	TC
BB24427	TC	TT	TC	TC	TC
BB24428	TC	, TT	TC	TC	CC
BB24429	TC	TT	TC	TC	CC
BB24430	TC	TT	TC	TC	T¢
BB24431	TC	<u> </u>	TC	TC	CC
BB24432	TC	TT	TC	TC	CC
BB24433	TC	<u> </u>	TC	TC	CC
BB24434	TC	<u>TT</u>	TC'	. TC	ÇÇ
BB24435	TC	<u> </u>	TC	TC	CC
BB24436	<u>TT</u>	<u>FL</u>	<u>cc</u>	CC	TC
BB24437	TÇ	<u>TT</u>	TC	TC	TC
BB24438	TC	<u> 11</u>	TC	TC	TC
BB24439	TC'	<u> </u>	TC	TC	CC
BB24440	TÇ	· <u> </u>	TC	FL	TC
BB2 <del>4444</del>	TC	TF -:	TC ·	TC	ÇĊ
BB24448	T	<u>FL</u>	CC	<u> </u>	FL
BB24461	TC	<u>T</u>	TC	TC	CC
BB24462	TT	FL	ÇĊ	CC.	TC
BB24463	TC	TT	TC	TC	ČČ
BB24464	TC	TT	TC	TC	CC
BB24465	TC	T	TC	TC	TC
BB24466	TC	. <b>TT</b>	TC	FL	TC
BB24467	TT ·	FL	CC	ÇĊ	TC
. BB24468	TC	17	TC	FL	TC
BB24469	TÇ	TT	TÇ	TO	CC
BB24470	TT ·	FL	CC	CC	TC
BB24471	TC	TT	TC	TC	TC
BB24472	TC	T	TC	TC	TC
BB24473	TC	TT	TC	TC	TÇ
BB24474	TC	<u> </u>	TC	TC	TC
BB24475	TÇ	TT	TÇ	TC	TC
BB24476	TC	77	TÇ	· TC	CC
BB24477.	TC ·	11	TC	TC	TĊ
BB24478	TC	TT	TÇ	TC	TÇ
BB24479	TC	17	TC	TG	TÇ
BB24480	TC	TT ,	TC	TC	TC

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BB24483	T	FL TT	CC	TC	TC
BB24484	TC	<b>TT</b>	TÇ CC	FL	TC
BB24485	TT	'FL	, CC ·		
BB24486	TT	FL	CC ·	CC	TC
BB24487	TC	TT	TC	TC	TC
BB24488	TG	<u>TT</u>	TC	TC	TC
BB24489	TC	<del>11</del>	TC	TC TO	TC
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BB24493	T	FL	CC	ÇC .	TC
BB24494	FL	FL	FL	FL	FL
BB24495	TC	<u> </u>	TÇ	TC	TC
BB24496	TÇ	<b>TT</b>	TC	. TČ	TC
BB24497	TC .	TT	TC	TC	CC
BB24499	TC	· π	TC	TC	TC
BB24504	TC	<u>TT</u>	TC	· TC	TC
BB24505	TC	TT	TC	TC	TC
BB24506	TC	<u> </u>	TC	TC	CC
BB24507	TC	<u> </u>	T¢.	TÇ	CC
BB24512	TT	FL	CC	СС	TC
BB24513	TC		TC	FL TO	TC
BB24516	TC	77	TC	TC.	CC.
BB24517	<u> </u>	· FL	CC	CC	TC
BB24518	TC	TT	TC	TC	T¢
BB24519	TC	TT	· TC	TC TC	. CC
BB24522	TC	T	TC	FL	FL
BB24523	FL TC	FL TT	FL TC	TC	CC
BB24524 BB24525	TG	ŤŤ	TC.	FL	TC
BB24526	тс	1_L	TC	TC	CC
BB24527	1 11	FL	CC	cc	TÇ
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BB24529	TÇ	TT	TC	TC	TÇ
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BB24531	FL	FL	FL	FL	FL
BB24532	TC	TT	TC	TÇ	CC
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BB24534	TC	TT	TC	FL	TC
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BB24540	TC	TT	TC	TC	TC
BB24541	TÇ	TT	TC	FL	TC
BB24542	TC	T	TC	TC	CC
BB24543	TÇ	TT	TÇ	TG	CC
BB24547	FL	FL'	FL	FL	FL
BB24548	TT	FL	CC	CC	TC
BB24549	ग	FL	CC	CC	FL
BB24550	TC	TT	T¢	TC ·	TC
BB24552	ТС	77	T¢	TÇ	TC
BB24553	TC	TT	TC	TÇ	CC
BB24554	TC	TT	TC	FL	TC
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18	17	17	15	16	16
354	355	355	357	356	356
95.16%	95.43%	95.43%	95.97%	95.70%	95,70%
0	O	O	348	112	263
28	1	2	7	155	89
326	354	353	2	89	4
3.95%	0.14%	0.28%	98.46%	53.23%	86,38%
96.05%	99.86%	99.72%	1.54%	46.77%	13.62%

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	Sample FL	Pass Rate
FL.		Na 1117-a luli an impalant managa kata basa basa luta an ing an 1117-a luma an
CC	1	91.7%
TC	0	100.0%
, LC	Q	100.0%
TC	0	100.0%
TC	O	100.0%
TC	1	91.7%
FL	han being an hand the same the	
TC	1	91.7%
CC	O O	100.0%
TT	0	100.0%
TC	1	91.7%
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TC	1	91.7%
TC	0	100.0%
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TC	0	100.0%
TC	Q.	100.0%
CC	0	100.0%
TT	0	100.0%
TT	0	100.0%
TC	Q	100.0%
<b>TT</b>	0	100.0%
TC	0	100.0%
TC	1	91.7%
<u> </u>	0	100.0%
TT	0	100,0%
· TC	0	100.0%
TC	1	91.7%
TC	0	100.0%
CC .	. 2	83.3%
CC	0	100.0%
CC	1	91.7%
TC	0	100.0%
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TC TC	0	100.0%
TC	1	91.7%
TC	.1	91.7%
TC	1	91.7%
TC	0	100.0%
CC	1	91.7%
TC	0	100.0%
CC	0	100.0%
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الهاجية	I A	100'À\$ [

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TT TC	0	100.0%
TC	0	100.0%
TC	1	91.7%
TÇ	0 2	100.0%
Ή	1	83.3%
CC		91.7%
TÇ	0	100.0%
CC	0	100.0%
TC	0	100.0% 100.0%
CC	4	91.7%
TC		91.7% 91.7%
FL		a fall plante property and the control
ΤĊ	Q (8), 東京 西部 福 墨語 智利 明朝	100.0%
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TC	ŏ	100.0%
CC	Ō	100.0%
TC	0	100.0%
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CC	] 1	91.7%
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7.7	1	91.7%
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CC	0	100.0%
TT	Aceta controlleron Sales e elemento e en el	100.0%
FL		
TC	0	100.0%
T	1	91.7%
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TC	1	91.7%
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cc	0	100.0%
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cc	o	100.0% 100.0%
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11	ő	100.0%
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TT	ŏ	100.0%
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TC	1	91.7%
CC	0	100.0%
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FL	Control of the contro	
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TC	2	83.3%
CC	0	100.0%
CC	O	100.0%
II	Ó	100.0%
TC ·	1	91.7%
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FL		
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TC	1	91.7%
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τċ	o	100.0%
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TC	4	66.7%
TC	1	91.7%
TC	0	100.0%
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CC	0	100.0%
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TT	1	91.7%
TT	0	100.0%
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TT	1 1	91.7%

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TO 1		
TC	0	100.0%
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TC	0	100.0%
17	0	100.0%
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CC	1	91.7%
TG	0	100.0%
TT	0	100.0%
TC	0	100.0%
TC	Q	100.0%
FL	reinengen schlieben fra bei geben in	
CC	]	91.7%
TC	1	91.7%
CC	1	91.7%
TC	1	91.7%
TT	0	100.0%
TC	2	83.3%
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0 6 DECEMBER 2005 06-12.05

- 69 .<del>-</del>

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TC	ő	100.0%
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CC	0	100.0%
TC	0	100.0%
TC	2	83.3%
CC	1	91.7%
TC	1	91.7%
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TC	1	91.7%
CC	. 0	100.0%
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CC	0.	100,0%
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TT	0	100.0%
TC	0	100.0%
TT	2	83.3%
CC.	0	100.0%
CC	J O	100.0%
CC	0	100.0%
TC	1	91.7%
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TC	3 0	75.0%
TC ·	0	100.0%
cc	2 0	83.3%
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TC	0	100.0%
<u>1</u>	0	100.0%
TC	a	100.0%
· CC	0	100.0%
TC	O STATE MATERIAL A	100.0%
FL FL	transfer in the State of the st	A A
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CC	0	100.0%
TC	1 1	91.7%
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TT	0	100.0%
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cc		100.0%
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T	1 1	91.7%
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TC	0	100.0%
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TC	0 0	100.0%
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FL		TO TAN
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FL	12	0.0%
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